Electronic Supplementary Material (ESI) for RSC Advances

Supplementary material

Non–specific clustering of Histidine tagged Green Fluorescent Protein mediated by surface interactions: the collective effect in the protein–adsorption behaviour

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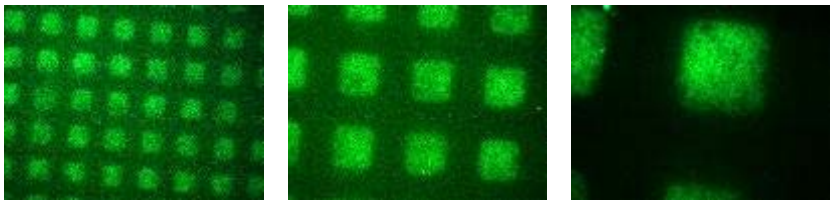
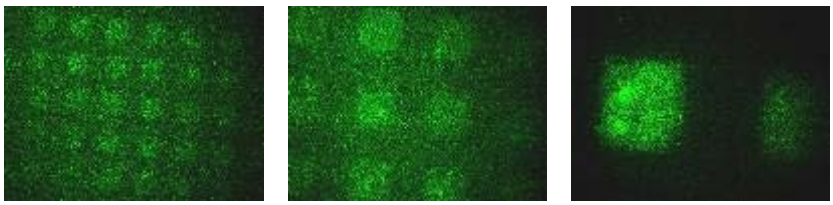
Fluorescence Microphotographs

Figure S1. Micropatterned surfaces functionalized with NTA after exposure to the His–tagged GFP. The same fragment of chip observed under increased microscope magnifications.
a) NTA–functionalized internal area of squares loaded with nickel(II) ions. Samples were incubated overnight at ambient temperature and subsequently washed with PBS buffer.



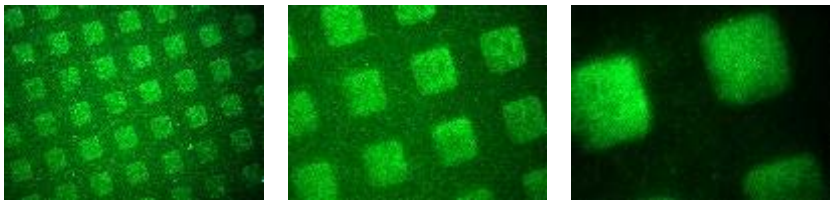
Magnification: 100x 200x 400x

b) Micropatterned surface after washing with buffer containing 250 mM imidazole (after 1 day of incubation and subsequent washing step).



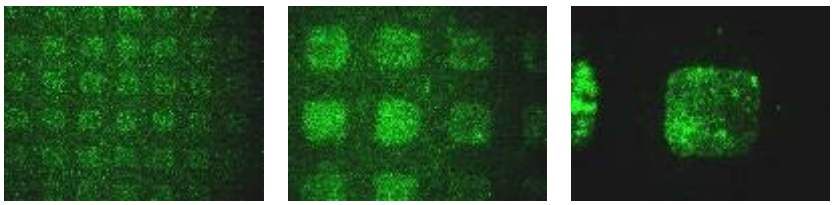
100x 200x 400x

Figure S2. Micropatterned surfaces functionalized with tacn–bis(formyl) after exposure to the His–tagged GFP. The same fragment of chip observed under increased microscope magnifications.
a) Tacn–bis(formyl)–functionalized internal area of squares after loading with nickel ions. Samples were incubated overnight at ambient temperature and subsequently washed with PBS buffer.



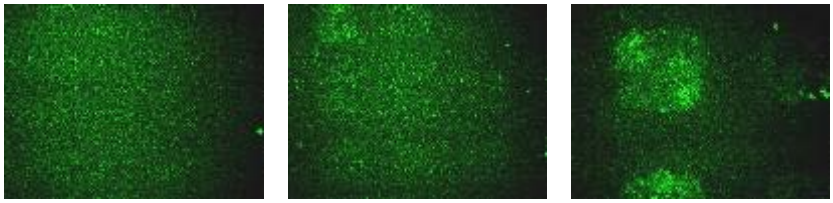
100x 200x 400x

b) Micropatterned surface after washing with buffer containing 250 mM imidazole (after 1 day of incubation and subsequent washing step).



100x 200x 400x

c) Micropatterned surface after washing with buffer containing 200 mM EDTA (after 5 min of incubation and subsequent washing step).



100x 200x 400x

d) His_6–GFP deposited on the micropatterned surface presenting tacn–bis(formyl) without nickel ions (after overnight incubation and subsequent washing step).

100x 200x 400x

Figure S3. Sequence of microphotographs – from 0 s to 60 s after loading of His–GFP on a whole surface of chip functionalized with tacn-bis(formyl) (without nickel ions) in internal area of squares. Protein is deposited directly under fluorescent microscope objective and observed immediately. Images are arranged in a series of pictures taken at a specific time (magnification 100x).

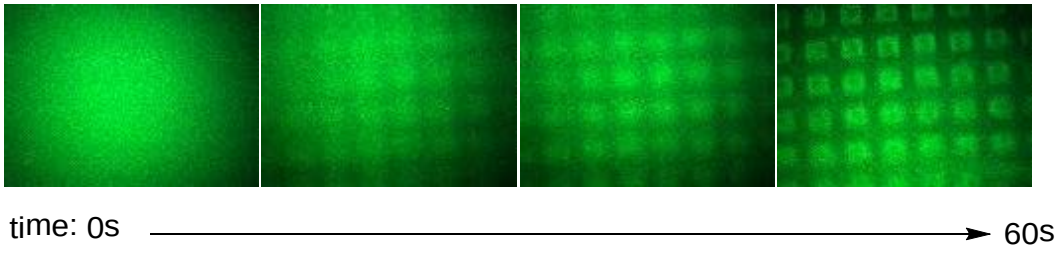
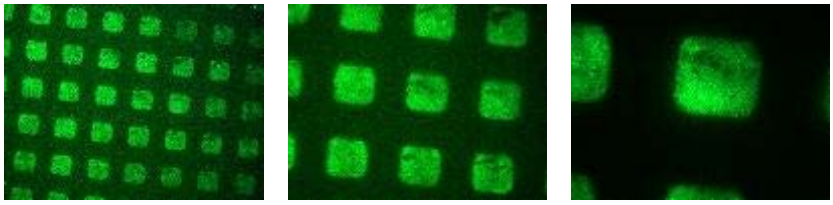


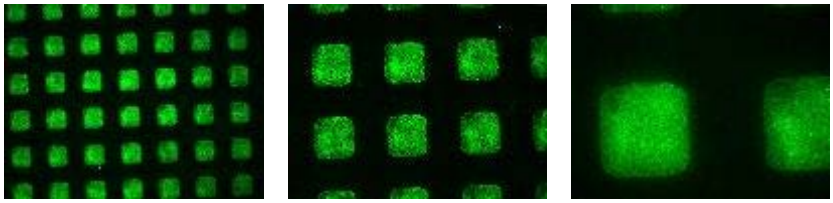
Figure S4. Micropatterned surfaces functionalized with N–hydroxysuccinimide ester after exposure to GFP or to fluorescently labeled proteins. The same fragment of chip observed under increased microscope magnifications.

a) His–GFP covalently bound to the micropatterned surface; images were acquired after overnight incubation and subsequent washing of chips with PBS buffer.



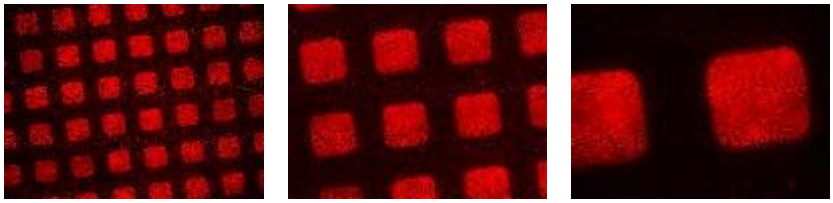
100x 200x 400x

b) FITC–Con A covalently bound to the micropatterned surface; images were acquired after overnight incubation and subsequent washing of chips with PBS buffer.



100x 200x 400x

c) Texas Red labeled BSA covalently bound to the micropatterned surface; images were acquired after overnight incubation and subsequent washing of chips with PBS buffer.



100x 200x 400x